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Claims

[c1] 1.A method of evaluating a test agent for the ability to modulate a parameter of heart function in a mammal, the method comprising:
(a) contacting a zebrafish heart with a test agent;
(b) evaluating a parameter of heart function in the zebrafish heart; and
(c) correlating the effect of the agent on the parameter of heart function in the zebrafish with a predicted effect on heart function in a mammal.

[c2] 2.The method of claim 1, further comprising generating a dataset correlating a value for the evaluated parameter with cardiotoxicity or probability of cardiotoxicity of the agent.

[c3] 3.The method of claim 1, wherein the parameter of heart function is heart rate.

[c4] 4.The method of claim 1, wherein the parameter of heart function is ejection fraction, contraction fraction, conduction velocity, repolarization, or Q-T interval.

[c5] 5.The method of claim 1, wherein the zebrafish is a wild-type zebrafish larva.

[c6] 6.The method of claim 1, wherein the zebrafish comprises a transgene comprising a heart-specific regulatory region operably linked to a nucleotide sequence encoding a fluorescent polypeptide.

[c7] 7.The method of claim 6, wherein the heart-specific regulatory region comprises SEQ ID NO:1.

[c8] 8.The method of claim 1, wherein the test agent causes an arrhythmia in the zebrafish heart.

[c9] 9.The method of claim 1, wherein the test agent is administered to the culture media of the zebrafish.

[c10] 10.The method of claim 1, wherein the test agent is injected into the zebrafish.

[c11] 11.The method of claim 1, wherein the zebrafish is a zebrafish larva.

[c12] 12.The method of claim 1, further comprising contacting the zebrafish heart with a second test agent.

[c13] 13.The method of claim 1, wherein the zebrafish has a genetic alteration in one or more genes related to heart function.

[c14] 14.The method of claim 1, wherein the method is performed in an array format.

[c15] 15.The method of claim 1, further comprising contacting the zebrafish with a dye.

[c16] 16.The method of claim 1, further comprising permeabilizing the zebrafish.

[c17] 17.The method of claim 1, wherein the test agent is evaluated in combination with a second test agent.

[c18] 18.The method of claim 1, wherein the test agent is a small molecule.

[c19] 19.The method of claim 1, wherein the test agent is a protein, DNA or RNA molecule.

[c20] 20.A method of determining if a test agent is cardiotoxic in a mammal, the method comprising:
contacting a developing zebrafish with a test agent;
measuring a parameter of heart contractility in the zebrafish, and
identifying a test agent that causes an abnormality in heart contractility in the zebrafish as a cardiotoxic agent in a mammal.

[c21] 21.The method of claim 20, wherein the parameter of heart contractility is heart rate or QT interval.

[c22] 22.The method of claim 20, wherein the abnormality is

arrhythmia.

[c23] 23.The method of claim 20, further comprising generating a dataset correlating a value for the parameter of heart contractility with cardiotoxicity or probability of cardiotoxicity of the agent.

[c24] 24.The method of claim 20, wherein the zebrafish is a wild-type zebrafish larva.

[c25] 25.The method of claim 20, wherein the zebrafish comprises a transgene comprising a heart-specific regulatory region operably linked to a nucleotide sequence encoding a fluorescent polypeptide.

[c26] 26.The method of claim 25, wherein the heart-specific regulatory region comprises SEQ ID NO:1.

[c27] 27.The method of claim 20, wherein the test agent is administered to the culture media of the zebrafish.

[c28] 28.The method of claim 20, wherein the test agent is injected into the zebrafish.

[c29] 29.The method of claim 20, wherein the zebrafish has a genetic alteration in one or more genes related to heart function.

[c30] 30.The method of claim 20, wherein the method is per-

formed in an array format.

[c31] 31.The method of claim 20, further comprising contacting the zebrafish with a dye.

[c32] 32.The method of claim 20, further comprising permeabilizing the zebrafish.

[c33] 33.The method of claim 20, wherein the test agent is evaluated in combination with a second test agent.

[c34] 34.The method of claim 20, wherein the test agent is a small molecule used or being considered for use as a pharmaceutical agent.

[c35] 35.The method of claim 20, wherein the parameter of heart contractility is measured by recording the zebrafish heartbeat and analyzing the recording.

[c36] 36.The method of claim 20, wherein the parameter of heart contractility is measured by determining an average pixel intensity or density throughout a specified region of the heart for a given time interval, and measuring the time between peaks of the intensity or density.

[c37] 37.The method of claim 20, wherein the parameter of heart contractility is measured by performing an EKG on the zebrafish.

[c38] 38.The method of claim 25, wherein the parameter of heart contractility is measured by scanning the zebrafish to identify a fluorescent region whose maximum intensity is above a control value; optionally recording the identified region for a specified time; calculating the average intensity through time for the fluorescent regions; and generating a dataset of the average intensity through time for the fluorescent region.

[c39] 39.The method of claim 26, wherein the parameter of heart contractility is measured by scanning the zebrafish to identify a fluorescent region whose maximum intensity is above a control value; optionally recording the identified region for a specified time; calculating the average intensity through time for the fluorescent regions; and generating a dataset of the average intensity through time for the fluorescent region.

[c40] 40.A method of evaluating the effect of a plurality of compounds on a parameter of heart contractility in a mammal, the method comprising:
contacting a developing zebrafish heart with a plurality of compounds,
evaluating a parameter of heart contractility in the zebrafish; and
correlating the effect of the plurality of compounds on the parameter of heart function in the zebrafish heart

with a predicted effect on a mammalian heart.

[c41] 41.The method of claim 40, wherein the parameter is heart rate or QT-interval.

[c42] 42.The method of claim 40, wherein the parameter is ejection fraction, repolarization, or conduction velocity.

[c43] 43.The method of claim 40, wherein the plurality of compounds is contacted simultaneously.

[c44] 44. The method of claim 40, wherein the plurality of compounds is contacted separately.

[c45] 45.The method of claim 40, wherein one of the plurality of compounds is a hormone.

[c46] 46.The method of claim 40, wherein the zebrafish comprises a transgene comprising a heart-specific regulatory region operably linked to a nucleotide sequence encoding a fluorescent polypeptide.

[c47] 47.The method of claim 40, wherein the test agent is a small molecule.

[c48] 48.The method of claim 40, wherein the parameter of heart contractility is measured by recording the zebrafish heartbeat and analyzing the recording.

[c49] 49.The method of claim 40, wherein the parameter of

heart contractility is measured by determining an average pixel intensity or density throughout a specified region of the heart for a given time interval, and measuring the time between peaks of the intensity or density.

[c50] 50.The method of claim 40, wherein the parameter of heart contractility is measured by performing an EKG on the zebrafish.

[c51] 51.The method of claim 46, wherein the parameter of heart contractility is measured by scanning the zebrafish to identify a fluorescent region whose maximum intensity is above a control value; optionally recording the identified region for a specified time; calculating the average intensity through time for the fluorescent regions; and generating a dataset of the average intensity through time for the fluorescent region.

[c52] 52.A method of evaluating the effect of a plurality of different treatments, the method comprising:
(a) providing an array of a plurality of individual regions, wells or addresses, each region, well or address of the plurality comprising a zebrafish larva being provided with a test treatment that differs from those at other regions, wells or addresses of the plurality; and
(b) evaluating a parameter of heart contractility of the zebrafish at each of the plurality of regions, wells or ad-

dresses, thereby evaluating the effect of a plurality of different treatments.

[c53] 53.The method of claim 52, wherein the plurality of different treatments comprises a plurality of different compounds.

[c54] 54.The method of claim 52, wherein the plurality of different treatments comprises a compound at a plurality of different concentrations or dosages.

[c55] 55.The method of claim 52, wherein the plurality of different treatments comprises a first compound in combination with a plurality of different second compounds.

[c56] 56.The method of claim 52, wherein each the plurality of zebrafish larvae comprises a transgene comprising a heart-specific regulatory region operably linked to a nucleotide sequence encoding a fluorescent polypeptide.

[c57] 57. The method of claim 52, wherein the parameter of heart contractility is measured by recording the heartbeat of the plurality of zebrafish and analyzing the recording.

[c58] 58.The method of claim 52 wherein the parameter of heart contractility is measured by determining an average pixel intensity or density throughout a specified re-

gion of the heart of each of the plurality of zebrafish for a given time interval, and measuring the time between peaks of the intensity or density.

[c59] 59.The method of claim 56, wherein the parameter of heart contractility is measured by: scanning the array to identify each of a plurality of fluorescent regions whose maximum intensity is above a control value; optionally recording each of the identified regions for a specified time; calculating the average intensity through time for each of the plurality of fluorescent regions; and generating a dataset of the average intensity through time for each of the plurality of fluorescent regions.

[c60] 60.The method of claims 53, wherein the plurality of different compounds is a plurality of different small molecules.

[c61] 61.A method of identifying a gene that affects a drug response; the method comprising:
providing a test zebrafish having a genetic alteration in a gene;
contacting the test zebrafish with a drug; and
evaluating the heart rate of the test zebrafish, wherein if the heart rate of the test zebrafish, compared to a control zebrafish, is increased or decreased, the gene is identified as a gene that affects a drug response.

[c62] 62.The method of claim 61, wherein the test zebrafish is genetically engineered.

[c63] 63.The method of claim 61, wherein the test zebrafish has decreased heart rate in response to the drug compared to a wildtype zebrafish.

[c64] 64. The method of claim 61, wherein one or both of the test zebrafish and the control zebrafish comprise a transgene comprise a heart-specific regulatory region operably linked to a nucleotide sequence encoding a fluorescent polypeptide.

[c65] 65.An isolated nucleotide sequence comprising a regulatory region comprising SEQ ID NO:1 operably linked to a heterologous coding sequence.

[c66] 66.The nucleotide sequence of claim 65, wherein the heterologous coding sequence encodes a protein not normally expressed in a cardiac cell.

[c67] 67.The nucleotide sequence of claim 65, wherein the heterologous coding sequence encodes a fluorescent protein.

[c68] 68.The nucleotide sequence of claim 65, wherein the regulatory region is less than 8000 nucleotides in length.

[c69] 69.A vector comprising the nucleotide sequence of claim 65.

[c70] 70.A host cell comprising the vector of claim 69.